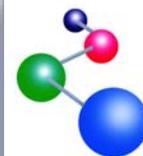


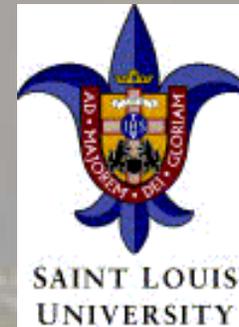
Midwest RCE Aerosol Biology and Small Animals Core



MRCE

Midwest Regional Center of Excellence
for Biodefense and Emerging Infectious Diseases Research

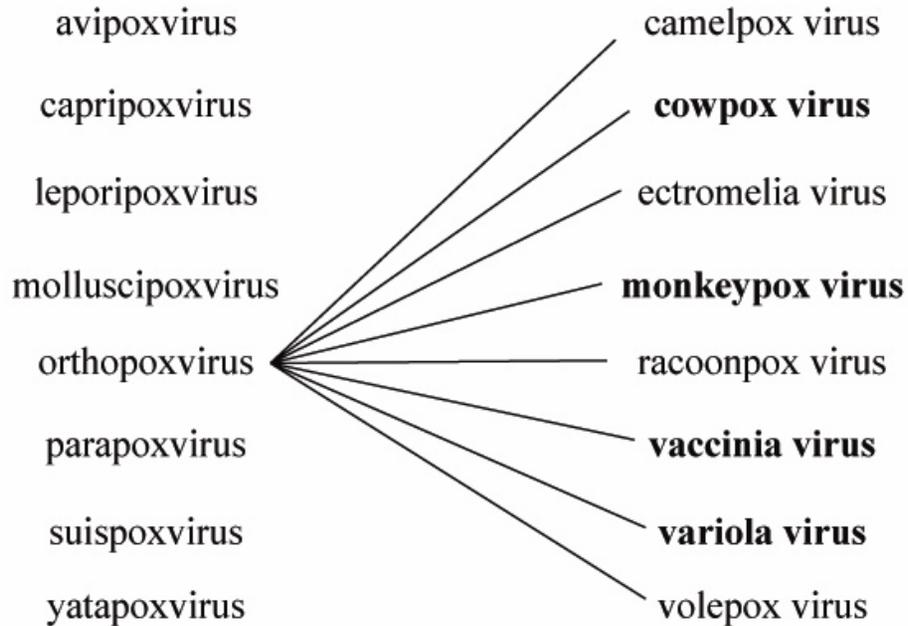
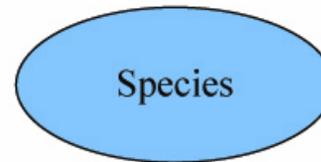
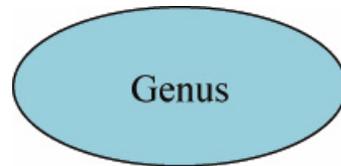
Regional Partners



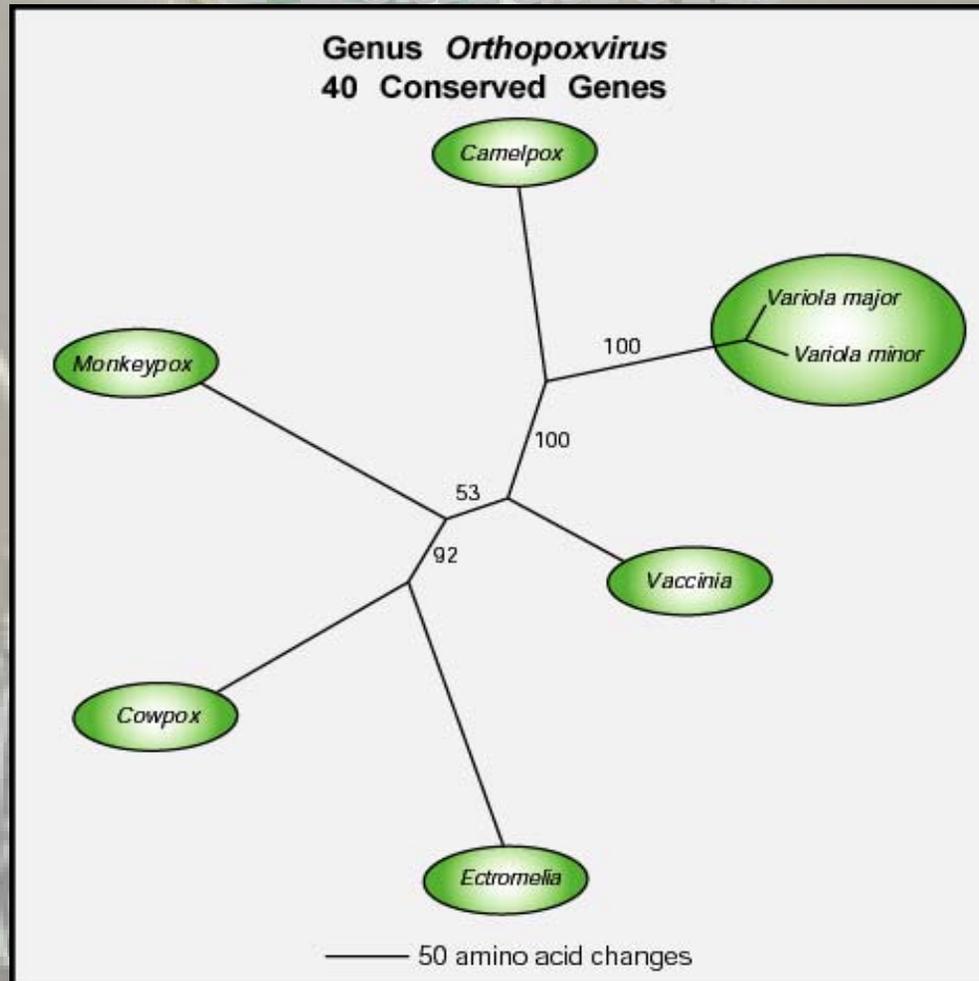
Mission

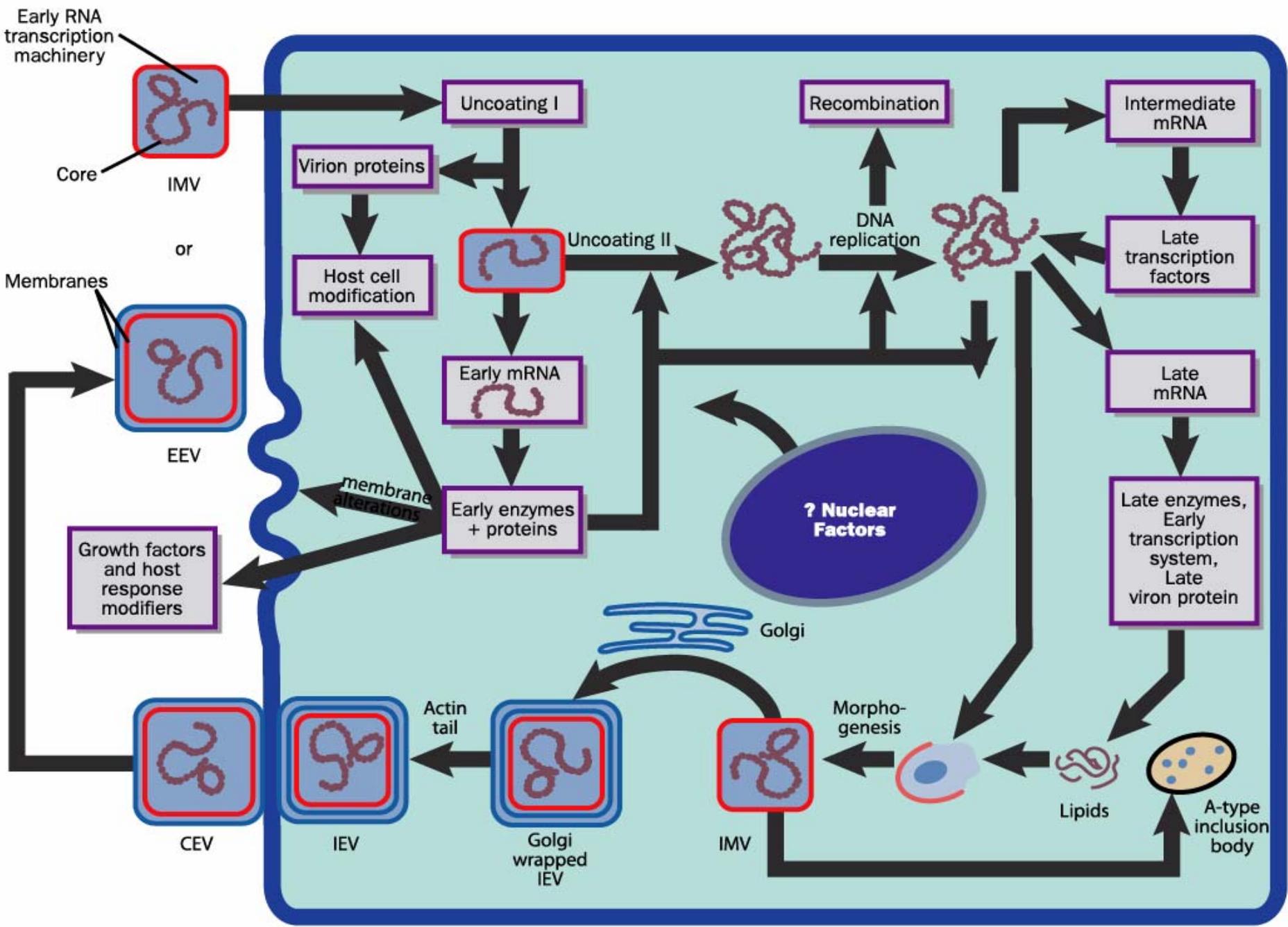
- Provide respiratory tract challenges for development of therapeutics and vaccines against smallpox and pneumonic plague
- Evaluate the delivery of therapeutics to the respiratory tract to study early intervention strategies
- Use the ectromelia model for the study of innate and acquired immunity to poxviruses
- Develop approaches for inactivation of bioparticles in the air

Poxvirus Taxonomy



Orthopoxvirus Phylogenetic Predictions





Early RNA transcription machinery

Core

or

Membranes

EEV

Growth factors and host response modifiers

CEV

IEV

Virion proteins

Host cell modification

Uncoating I

Uncoating II

Early mRNA

Early enzymes + proteins

Recombination

DNA replication

Intermediate mRNA

Late transcription factors

Late mRNA

Late enzymes, Early transcription system, Late virion protein

? Nuclear Factors

Golgi

Actin tail

Golgi wrapped IEV

IEV

Morphogenesis

IMV

Lipids

A-type inclusion body

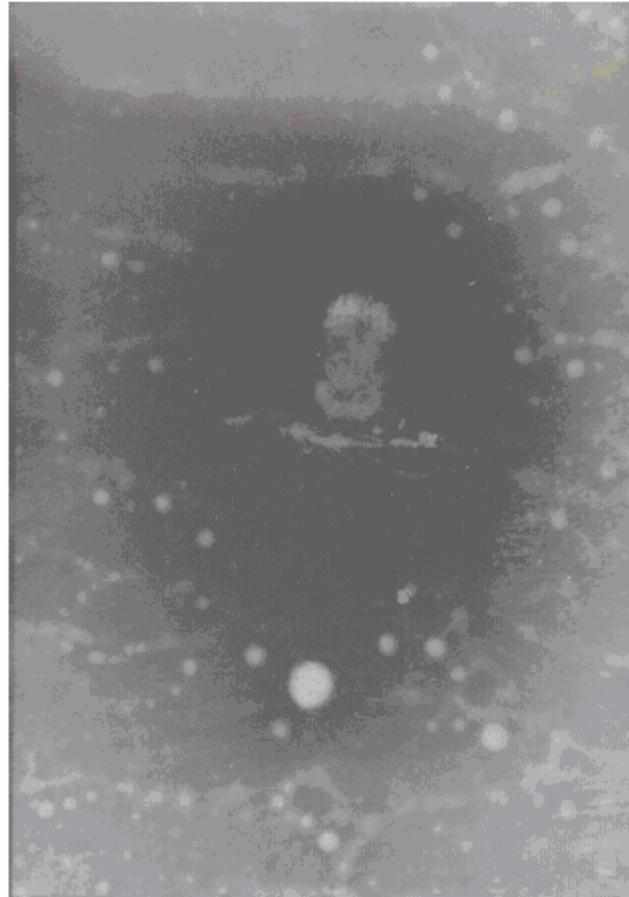
IMV

CEV

membrane alterations

Growth factors and host response modifiers

Negative-Stained Virion



Thin-section of Cowpox Virion

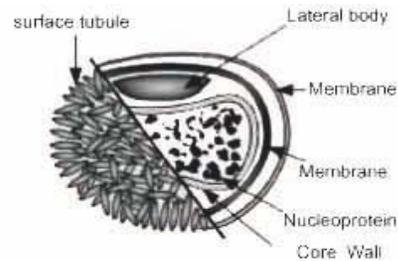
A



Immature
Particles

Virion

B



surface tubule

Lateral body

Membrane

Membrane

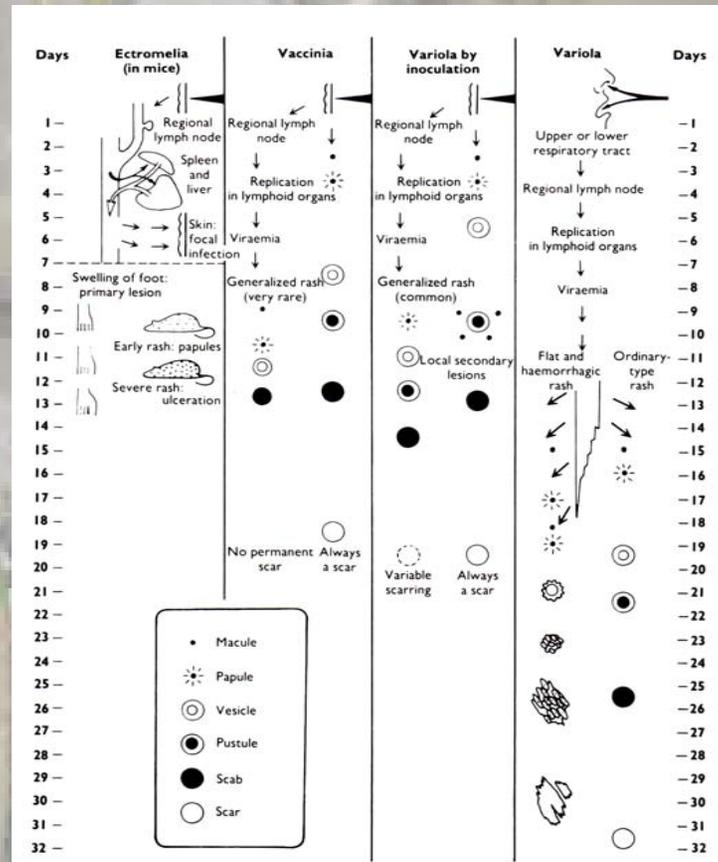
Nucleoprotein

Core Wall

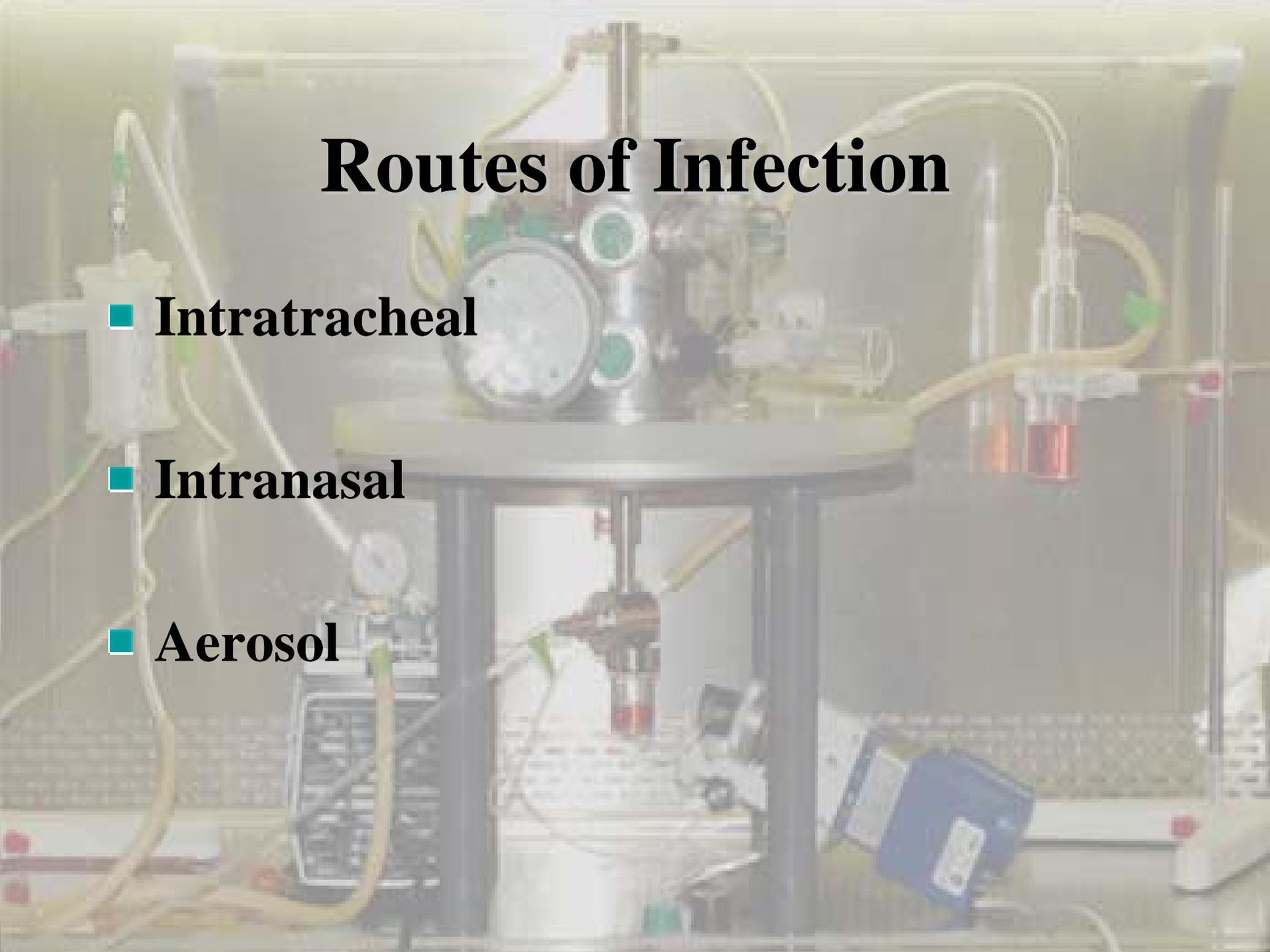
Small Animal Models for Poxvirus Respiratory Infection

- **Cowpox virus** $LD_{50} \sim 10^4$ PFU
- **Ectromelia virus** $LD_{50} < 50$ PFU
- **Monkeypox virus** limited models
- **Vaccinia virus** $LD_{50} \sim 10^5$

Pathogenesis of orthopoxvirus



Routes of Infection

A laboratory setup for studying routes of infection. The apparatus is mounted on a metal frame. At the top, a central chamber contains a mouse. This chamber is connected via yellow and white tubes to various components: a reservoir on the left, a vertical tube with a stopcock on the right, and a lower chamber containing a mouse. A blue power supply unit is visible at the bottom right. The background is a plain wall.

- **Intratracheal**

- **Intranasal**

- **Aerosol**

Intranasal Ectromelia Virus Infection of A/NCR mice

Virus Dose (PFU/mouse)¹	Mean time to Death	Mortality rate	LD₅₀² (PFU)
5,000	7±0.0	100%	
500	7.3±0.5	100%	
50	8±0.0	100%	0.3
5.0	9±0.0	100%	
0.5	10±1.4	50%	
0.05	10	25%	

¹Experiment C-97

²Reed and Muench

Aerosol Ectromelia Virus Infection of A/NCR mice

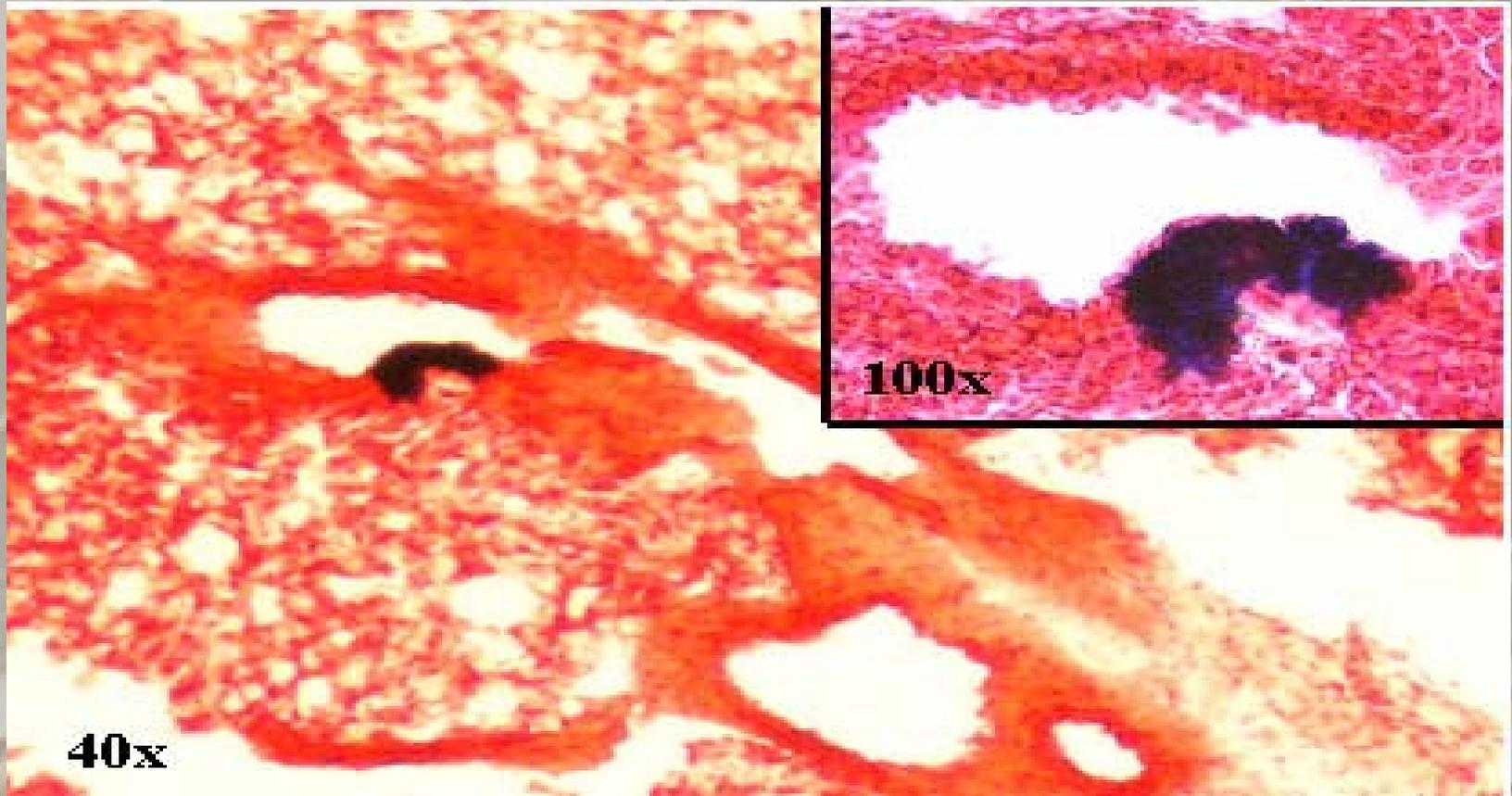
Presented Dose (PFU/mouse)¹	Mean time to Death	Mortality rate	LD₅₀² (PFU)	Seroconversion of survivors at T=21 days pi
1.9 x 10 ⁴	8.1±0.4	100%		N/A ³
1.3 x 10 ³	9.3±0.5	100%		N/A
63	10.2±1.3	67%	32	1/3
6.3	14	9%		0/7
0	N/A	0%		0/8

¹Experiment C-78

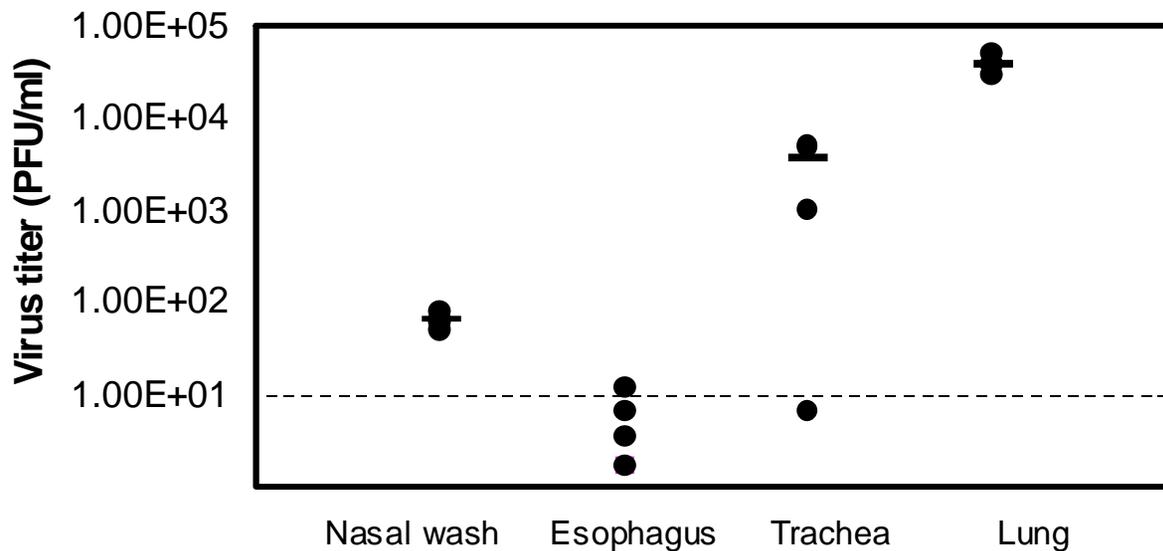
²Reed and Muench

³not applicable

Ectromelia Virus Focus of Infection in the Mouse Lung



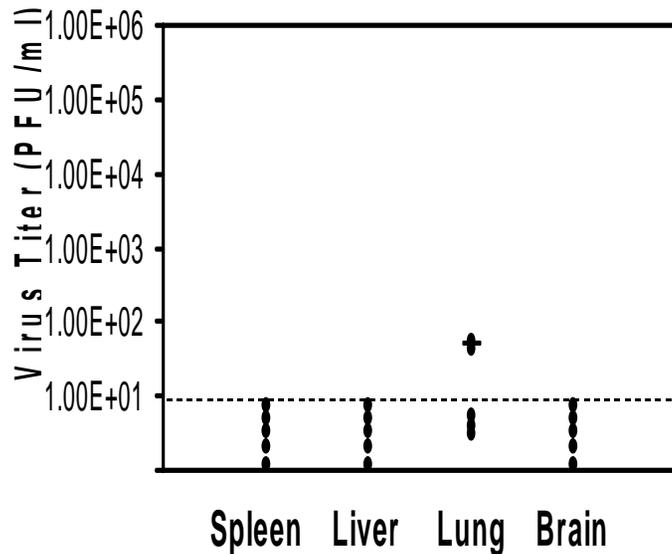
Tissue Infectivity Following Aerosol Infection



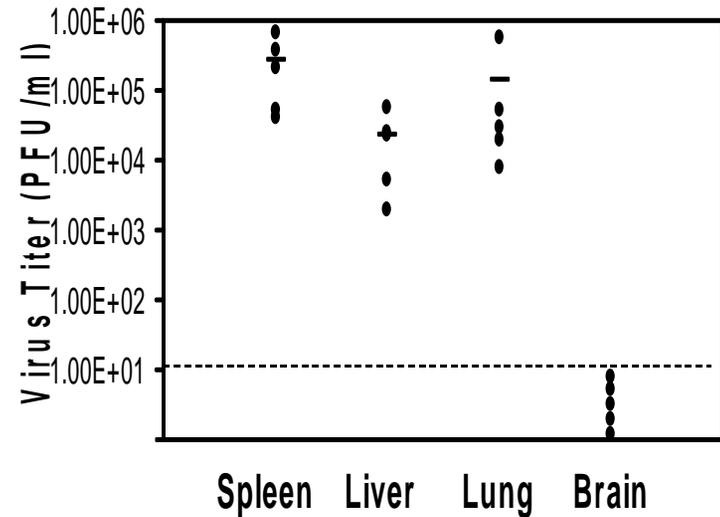
Presented aerosol dose 1.6×10^4 PFU ($1000 LD_{50}$), C-90

Low Dose Ectromelia Virus Aerosol Challenge of Vaccinated Mice

Dryvax Vaccinated mice



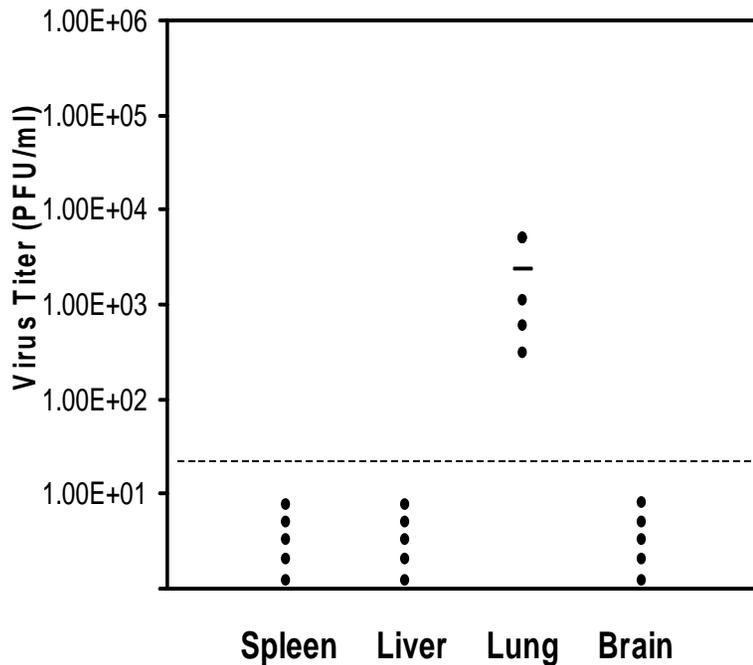
Control Mice



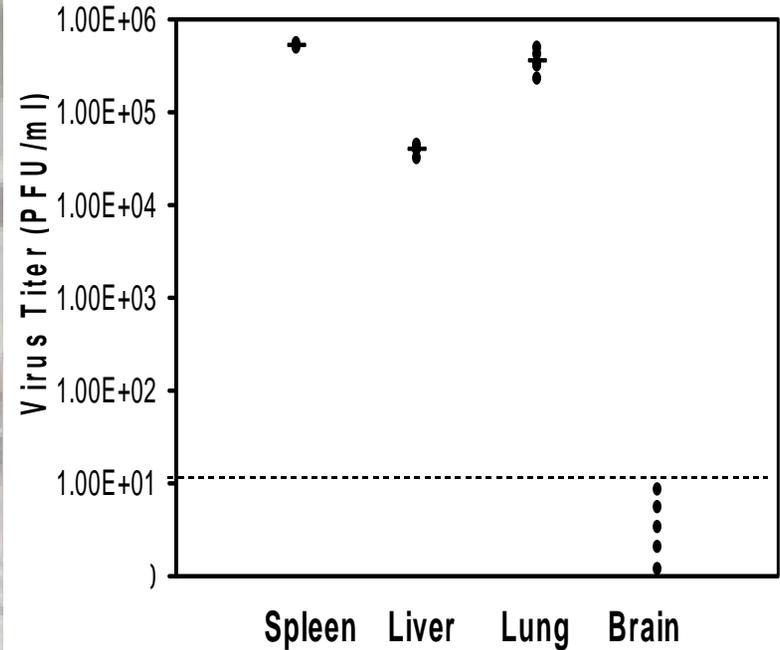
Presented aerosol dose 7.3×10^3 PFU (200 LD₅₀)

High Dose Ectromelia Virus Aerosol Challenge of vaccinated mice

Dryvax Vaccinated Mice



Control Mice



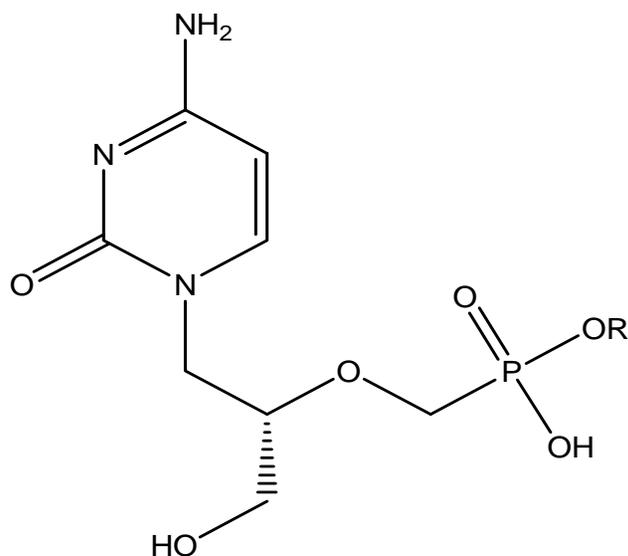
Presented aerosol dose 3.4×10^4 PFU (1000 LD₅₀)

Aerosolized Cidofovir Treatment of an Aerosol Cowpox Virus Infection

Mean Aerosol Dose and wt	Aerosol Cidofovir			Subcutaneous Cidofovir		
	Day Treated	Dose (mg/kg)	Survival	Day Treated	Dose (mg/kg)	Survival
5.4 x 10 ⁵ PFU/ 8.9 g	0	0.5-5	10/10*	0	25	7/10*
	1	0.5-5	9/10*	1	25	6/10*
	2	0.5-5	5/10*	2	25	5/10*
	Placebo	N/A	0/8			

From: M. Bray Antiviral Research 54 (2002) 1219-142

Structure of Cidofovir and Ether Lipid Esters



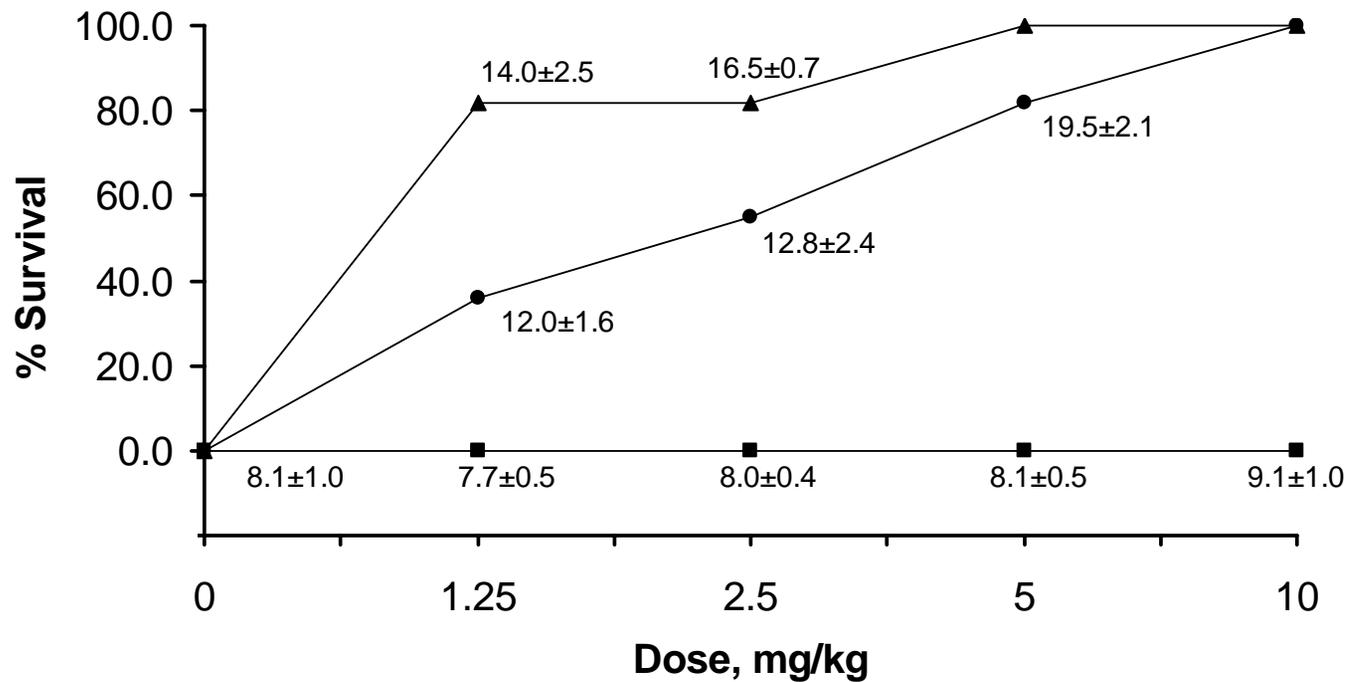
H
 $\text{CH}_3(\text{CH}_2)_{15}\text{O}(\text{CH}_2)_3$
 $\text{CH}_3(\text{CH}_2)_{17}\text{O}(\text{CH}_2)_2$
 $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_8\text{O}(\text{CH}_2)_2$
 $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_8\text{O}(\text{CH}_2)_3$

compound

cidofovir
hexadecyloxypropyl cidofovir, HDP-CDV
octadecyloxyethyl cidofovir, ODE-CDV
oleyloxyethyl cidofovir, OLE-CDV
oleyloxypropyl cidofovir, OLP-CDV

Efficacy of HDP-CDV and ODE-CDV

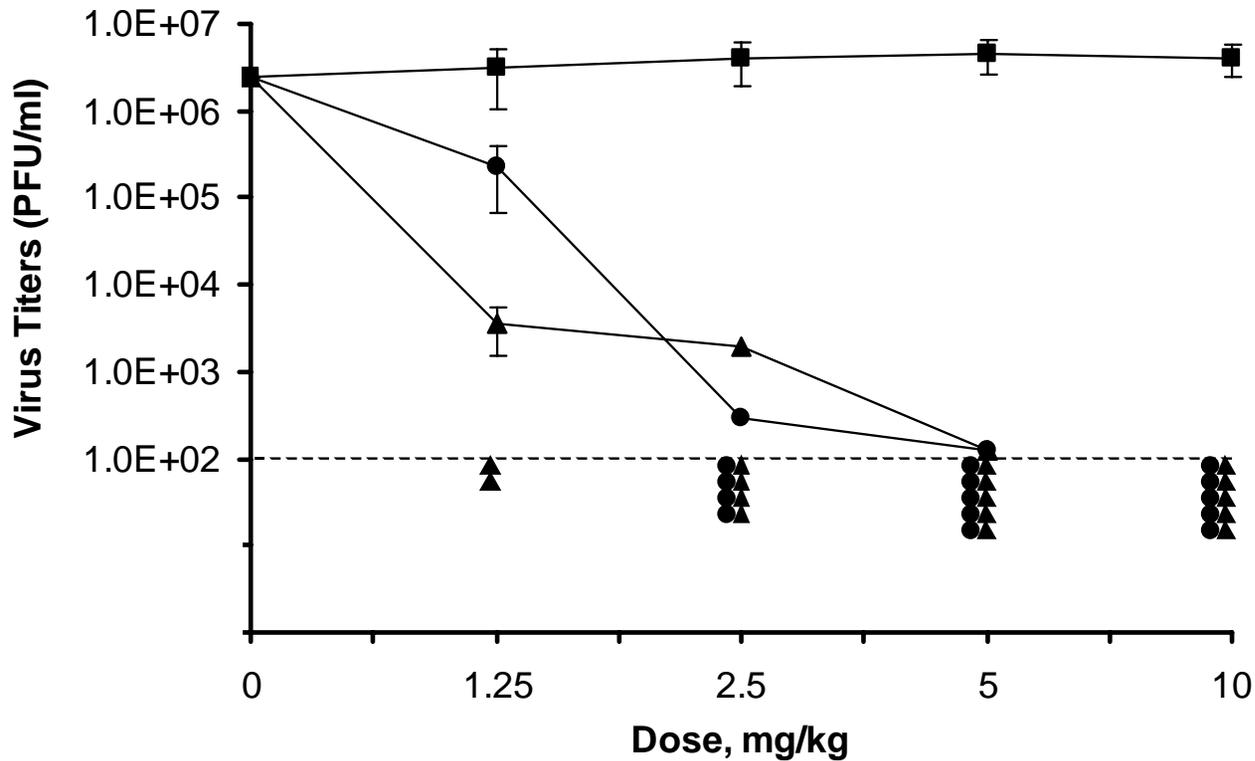
Survival



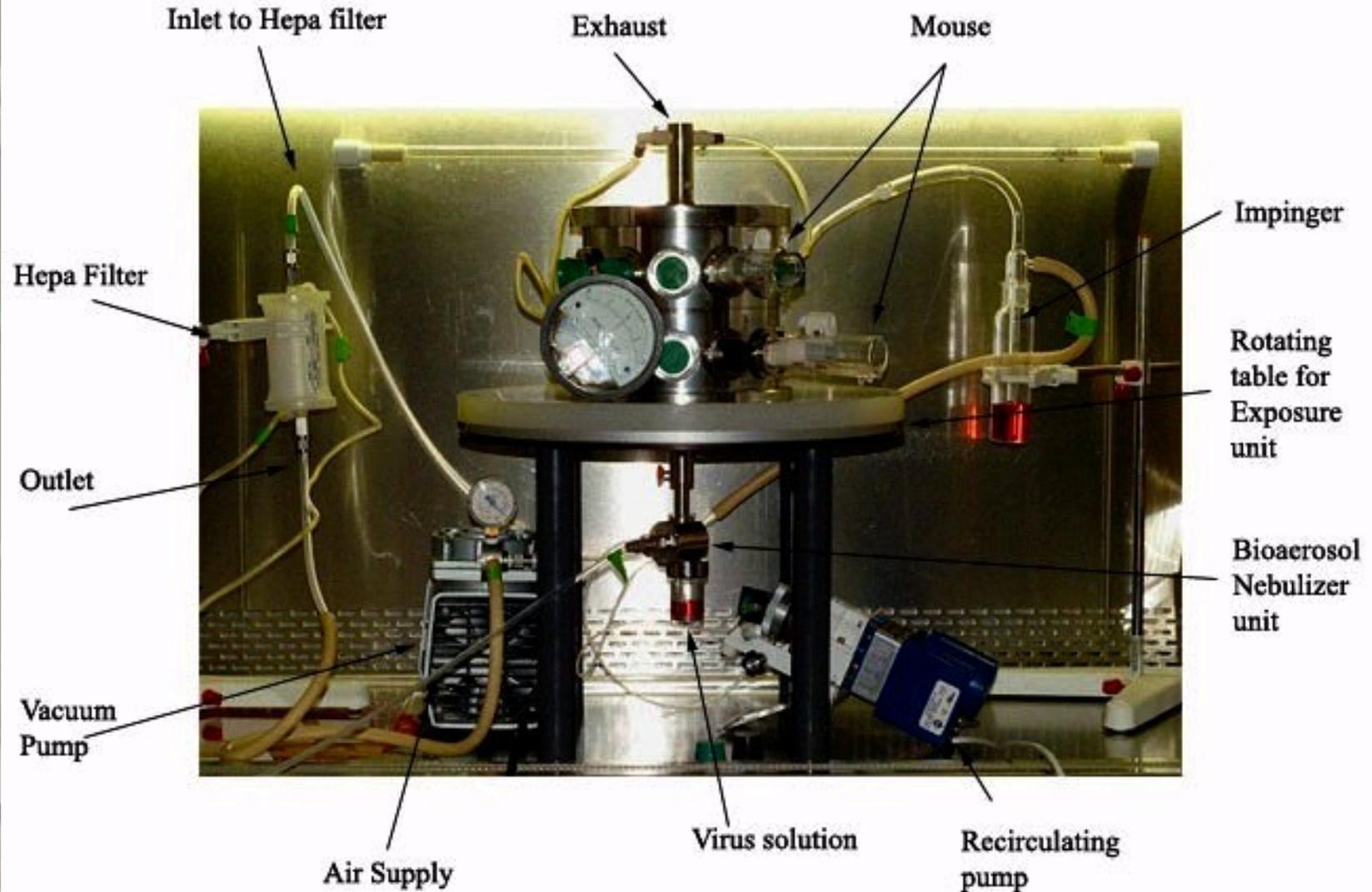
Presented aerosol dose 1.3×10^3 PFU (50 LD₅₀) C-50/52

Efficacy of HDP-CDV and ODE-CDV

Liver Titers



Nose-only Inhalation Exposure System



Collaborators

Saint Louis University

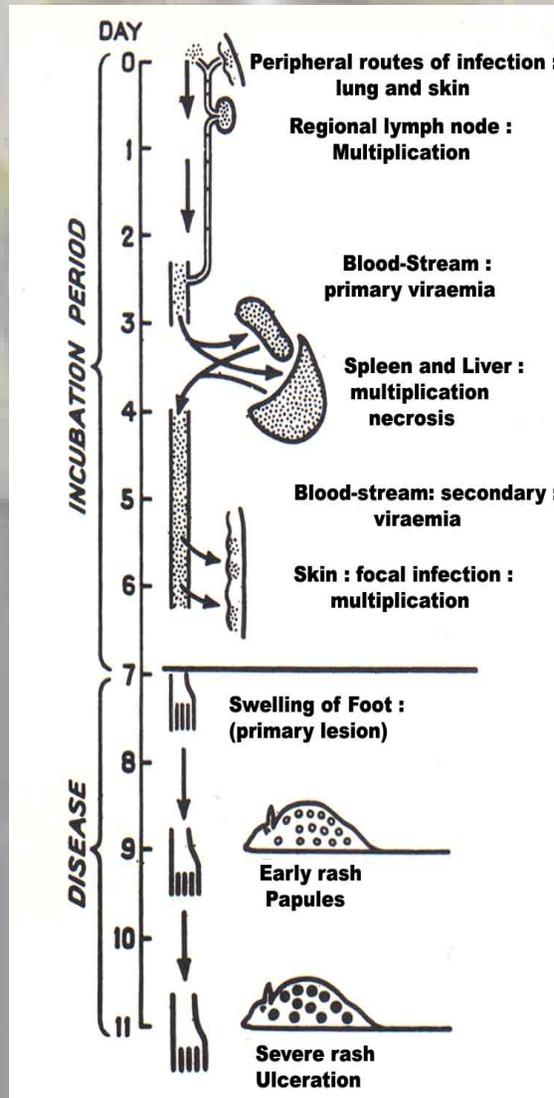
- Jill Schriewer
- Gelita Owens
- Mark Buller
- Roger Lewis
- Chad Roy

Washington University

- Pratim Biswas
- Da-Ren Chen
- Myong-Hwa Lee

Initial Funding from an Antiviral contract with NIAID

Mousepox



Tissue Infectivity Following Intranasal Infection

